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Oxidative Mapping of Alzheimer's Disease Amyloid Fibrils
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Introduction: Alzheimer's disease amyloid fibrils are conglomerates of a large number of Abeta peptide molecules (40-43 residues). Because of their size, and rapid formation, conventional NMR and X-ray structural methods cannot be applied to the fibrils. Using X-ray radiolysis methodology (Maleknia et al., 2001), amyloid fibril structure is being mapped. Carefully prepared fibril samples are radiolyzed, and then the oxidized Abeta is recovered via chemical solubilization. The oxidative pattern is then analyzed via liquid chromatography mass spectrometry using and ion-trap detector. Multiple rounds of mass spectrometry are necessary to fully map the sites of oxidation. Mapping data from these experiments is then combined with other chemical modification data (Iwata, Eyles & Lee, 2001), and will be used to construct atomistic models of amyloid fibril structure. This will set the stage to use the structure of the amyloid fibril as well as that of soluble Abeta (Zhang et al., 2000) as input structures for reaction path calculations (Ho & Straub(1997), Straub et al. 2001).

Methods and Materials: All peptides are synthesized by the Merrifield solid-phase methodology as described (Lee et al., 1995, Zhang et al., 2000). Peptides are purified by RP-HPLC and analyzed by MALDI or LC-ESI mass spectrometry. All X-ray foot-printing exposures were carried out at the NIH resource located at the NSLS, BNL. The experimental setup is found within Maleknia *et al*. (2001). Importantly, the duration of •OH exposure is directly proportional to the X-ray exposure time. Hence varying the exposure time alters the "dose" of radicals. Control of exposure is carried out with an electronic shutter (Vincent Associates, Rochester, NY). Typically 0.5 milliliter eppendorf tubes were held in thermo-regulated aluminum block. The sample volume in the bottom of the eppendorf is 10 microliters. Exposure is allowed until approximately 20 – 80% of the molecules remain un-oxidized. Three separate trials on test samples gave 40 – 50 milliseconds as a suitable exposure time. Immediately following exposure, samples are frozen on dry ice. Limited proteolysis and mass spectrometry are being performed within a few days of exposure to avoid any subsequent chemical decomposition.

Results: Thus far we have carried out preliminary structural mapping studies. In general, when soluble Abeta was exposed to synchrotron radiation, extensive oxidation was found within the entire molecule. However, for Abeta assembled into amyloid fibrils, little oxidation was detected within a fragment corresponding to residues 11-25 (EVHHQKLVFFAEDVG). In addition, there appears to be more oxidation within the first 10 residues (DAEFRHDSGY) compared to the last 14 residues (SNKGAIIGLMVGGVV). Further studies are underway to assign the oxidative modification to specific amino acid residues.

Conclusions: These preliminary studies, combined with other non-radiolytic data, show that we can successfully conduct chemical mapping on both the soluble and amyloid fibril forms of Abeta. With these data in hand the feasibility of successfully constructing an atomistic model of the amyloid fibrils based detailed experimental results deserves significant optimism.

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